NUCLEOTIDE AND/OR AMINO-ACID SEQUENCE CONTROLLING THE EXPRESSION OF A XYLANASE PROMOTER-OPERATOR NUCLEOTIDE SEQUENCE

Field of the invention

The present invention is related to a new nucleotide sequence controlling in trans the expression of a xylanase promoter-operator nucleotide sequence, the amino-acid sequence encoded by said new nucleotide sequence, the vector comprising said new nucleotide sequence and the cell, preferably a *Streptomyces* strain, transformed by said vector.

Background of the invention

In beer production, efficient hydrolysis of xylans and other saccharides is important because said compounds can be involved in production problems such as wort viscosity (Ducroo, P. & Frelon, P.G., *Proceedings of the European Brewery Convention Congress, Zurich*, 1989, 445; Viëtor, R.J. & Voragen, A.G.J., *Journal of the Institute of Brewing*, 1993, **99**, 243) and filterability and haze formation (Coote N. & Kirsop, B.H., *Journal of the Institute of Brewing*, 1976, **82**, 34; Izawa, M., Kano, Y. & Kanimura, M., *Proceedings Aviemore Conference on Malting, Brewing and Distilling*, 1990, 427).

In other areas, efficient hydrolysis of xylans and/or arabinoxylans is highly desirable as well. Examples include rye and wheat breadmaking processes, paper and pulp technologies (see US patent 5,116,746). It follows that a lot of research efforts have been devoted to the xylan hydrolysis enzymes due to their applications as described above.

Aims of the present invention

The aim of the present invention is to provide a method and system which improve the control upon the expression of nucleotide sequence encoding enzymes such as xylanase, as well as homologous or heterologous sequences of said enzymes whose transcription is also activated by a xylanase promoter-operator regulatory sequence.

A specific aim of the present invention is to provide such a method and system for improving enzymatic processes, especially for improving production of antibiotics, malting processes of cereals such as barley, sorghum and wheat, production of beers, of baked or extruded cereals products, animal feed stuff, the production of starch derived from syrups, sorbitol, xylose and xylitol, and for the improvement of paper and pulp technologies.

Summary of the invention

The present invention is related to a new nucleotide sequence 1 which controls the expression of any xylanase promoter-operator nucleotide sequence 2. Said control upon the activation of a xylanase promoter-operator nucleotide sequence 2 is advantageously obtained by trans-activation (said new nucleotide sequence 1 encoding a

trans-activated factor which controls the activation of said xylanase promoter-operator nucleotide sequence 2).

Therefore, the present invention is also related to said factor, preferably a peptidic factor 3 which is an activator and/or repressor encoded by said nucleotide sequence 1 and which controls positively and/or negatively the expression of a xylanase promoter-operator nucleotide sequence 2.

Advantageously, said factor could be present in a composition with other cofactors 4 that induce positively and/or negatively said mechanism.

Preferably, said cofactors 4 present in said composition are selected from the group consisting of glucose, xylan or a mixture thereof.

The Inventors have discovered unexpectedly that the presence of glucose induces a repressive mechanism upon the activation of a xylanase promoter-operator nucleotide sequence, while the presence of xylan induces a positive mechanism of said expression. The simultaneous presence of said two cofactors in a medium induces also positively the expression of a xylanase promoter-operator nucleotide sequence.

It is meant by "a xylanase promoter-operator nucleotide sequence", any nucleotide sequence 2 which cis-activates any nucleotide sequence 5 encoding a xylanase enzyme.

A classification of the xylanase enzymes in the categories F/10 and G/11 is described by Henrissart et al. (*Biochem. J.* 293, pp. 781-788).

Said xylanase promoter-operator nucleotide sequences comprise at least one 5 base pairs pattern : 5'-CGAAA-3'.

Preferably, said xylanase promoter-operator nucleotide sequence is the *Streptomyces sp.* strain EC3 xlnC

xylanase promoter-operator nucleotide sequence SEQ ID NO 2 also described by Giannotta F. et al. (*FEMS Microbiol. Letters* **142**, pp. 91-97 (1996)).

According to a preferred embodiment of the present invention, the isolated and purified nucleotide sequence according to the invention is a (DNA) sequence which presents more than 60%, advantageously more than 80%, preferably more than 90%, and more preferably more than 95%, homology (i.e. sequence identity) with the nucleotide sequence SEQ ID NO 1 or its complementary strand described hereafter.

According to another preferred embodiment of the present invention, said isolated and purified nucleotide sequence corresponds to the nucleotide sequence SEQ ID NO 1 or its complementary strand or a portion thereof; preferably a sequence having more than 100 nucleotides and encoding a peptide which still controls positively and/or negatively the expression of a xylanase promoter-operator nucleotide sequence.

Preferably, said sequence portion comprises at least the nucleotides of SEQ ID NO 3 or any nucleotide sequence encoding for its corresponding peptidic sequence.

According to a further preferred embodiment of the present invention, the terms "a portion of the nucleotide sequence SEQ ID NO 1 or its complementary strand" mean any kind of nucleic acid molecule (DNA, RNA, antisense nucleotide sequence, etc.) which is specific of SEQ ID NO 1, comprises more than 15 nucleotides (such as a probe or one or several primers), and which may be used to identify, reconstitute or block the transcription of said specific isolated and purified nucleotide sequence SEQ ID NO 1 or its complementary strand. Said identification, reconstitution or blocking is obtained with known techniques by the person skilled in the art, such as the

use of antisense RNA, specific labelled probe hybridisation or genetic amplification, preferably by PCR (as described in the US patent 4,965,188) or by LCR (as described by Landgren et al. (*Sciences* 241, pp. 1077-1080 (1988)).

Therefore, the present invention is also related to any nucleotide sequence which presents an homology (i.e. sequence identity) as above-described with SEQ ID NO 1, SEQ ID NO 3 or their complementary strands, or any nucleotide sequence which preferably allows an hybridisation with SEQ ID NO 1, SEQ ID NO 3 or their complementary strands under standard stringent hybridisation conditions, and which may encode the same or a similar amino-acid sequence due to the redundancy of the genetic code.

Exemplary standard stringent hybridisation conditions are as follows : hybridisation at 40 °C in 50% formamide, 5x SSC, 20 mMol sodium phosphate, pH 6.8, washing in 0.2x SSC at 50 °C. Variations in these conditions may occur based on the length and the GC nucleotide content of the sequence to be hybridised. Formula standard in the art are approved for determining exact hybridisation conditions such as the one described by Sambrook et al. (*Molecular Cloning : A Laboratory Manual, Cold Spring Harbor, Laboratory Press, Cold Spring Harbor, New York*, §9.47-9.51 (1989)).

Another aspect of the present invention is related to the amino-acid sequence encoded by said nucleotide sequence, and which present more than 60%, advantageously more than 80%, preferably more than 90%, more preferably more than 95% homology (i.e. sequence identity), with SEQ ID NO 2.

According to another embodiment of the present invention, the amino-acid sequence according to the

invention corresponds to the amino-acid sequence of SEQ ID NO 2 or any portion thereof having preferably more than 50 amino-acids and which is still capable of controlling (positively or negatively) in trans the expression of a xylanase promoter-operator nucleotide sequence.

Preferably, said portion is an amino-acid sequence that comprises at least the amino-acid sequence encoded by the nucleotide sequence SEQ ID NO 3 above-described.

It is meant by "controlling (positively and/or negatively) in trans the expression of a xylanase promoter-operator nucleotide sequence", the possibility for any nucleotide sequence 1 or any amino-acid sequence 3 encoded by said nucleotide sequence 1 to induce or reduce (preferably in the presence of the other cofactors 4 such as glucose and/or xylan) the expression of a xylanase promoter-operator nucleotide sequence 2 and obtain thereafter a control upon the cis-activation of a downstream nucleotide sequence 5 (for instance a gene encoding a xylanase enzyme) which is controlled in cis by said xylanase promoter-operator nucleotide sequence. The inducing or reduction of said expression is observed preferably by a positive or a negative modification of said cis-activation (for instance by an increasing or a decreasing of the synthesis of said xylanase enzyme by a cell). Said mechanism is also illustrated in the enclosed Fig. 3.

The present invention is also related to a nucleotide construct 6 comprising the isolated and purified nucleotide sequence 1 according to the invention, linked to a xylanase promoter-operator nucleotide sequence 2 and possibly any homologous or heterologous nucleotide sequence 5 of a gene encoding a xylanase enzyme, which is cis-

activated by said xylanase promoter-operator nucleotide sequence 2.

Another aspect of the present invention is related to the vector 7 comprising said isolated and purified nucleotide sequence 1 or the nucleotide construct 6 according to the invention. Advantageously, said vector 7 is a plasmid comprising the necessary elements (origin of replication ORI) for the transfection of said nucleotide sequence 1 or said nucleotide construct 6 into a cell, preferably into a *Streptomyces sp.* strain.

The vector according to the invention may comprise also other elements, such as a marker (thiostreptone = tsr) for the identification of a possible transformation by the vector according to the invention in said specific cell. The vector according to the invention can be also a shuttle vector comprising the necessary elements for the expression of said shuttle vector in *E. coli* and *Streptomyces sp.*

Another aspect of the present invention is related to the cell such as a gram-positive bacteria, preferably a *Streptomyces* strain, transformed by said vector 7 or by said shuttle vector, which allows the expression of the isolated and purified nucleotide sequence 1 according to the invention controlling the activation of the xylanase promoter-operator nucleotide sequence 2 present in said cell and therefore the transcription of any nucleotide sequence 5 which could be cis-activated by said xylanase promoter-operator nucleotide sequence 2.

The nucleotide construct 6, the vector 7 and/or the cell transformed by said vector as well as specific portions of the isolated and purified nucleotide sequence 1 according to the invention can be advantageously used in several industrial biochemical processes such as production of antibiotics, malting processes of cereals,

preparation of beers, baked or extruded cereals products, for the improving of animal feed stuff and for the improvement of paper and pulp technologies.

The products of the invention, possibly combined with the above-described cofactors, are advantageously present in a bioreactor, and will allow the controlled synthesis of proteins or peptides of interest or possibly avoid or reduce the synthesis of said proteins or peptides by specific cells in the above-identified biochemical industrial processes.

The various aspects of the present invention will be described in details in the enclosed non-limiting examples in reference to the following figures.

Brief description of the drawings

Figures 1 to 3 represent the steps for the construction of the vector according to the invention.

Detailed description of the invention

The alignment of various nucleotide sequences upstream xylanase gene in the strain *Streptomyces sp.* EC3, shows the presence of three repetitive units of five BP : 5'-CGAAA-3' observed among all xylanase sequences (except in the strain *Actinomadura sp.* which comprises only one repetitive unit).

In the specific strain *Streptomyces sp.* EC3, three boxes in the promoter-operator regions of 390 BP are defined : box 1 (B1) at -200 BP, box 2 (B2) at -210 BP and box 3 (B3) at -350 BP from the ATG codon. The box B3 is extremely conserved between the *Streptomyces* strain. (83% of identity of sequence upon 12 bases).

The identification of the repetitive consensus sequence is presented in the following table 1.

Table 1

Cons. B1	C G A A A C T G T T G A
Cons. B2	T T T C C G A A A G T T T G C C
Cons. B3	T C G A A A C T T T C G
Consensus	t **C G A A A** g c c

However, it seems that said consensus nucleotide sequence is not present in other known xylanase nucleotide sequence of other bacteria such as *Bacillus* strains.

The Inventors have discovered that the proteinic trans-activation factor according to the invention affects the regulation of said specific portions (B3 > B2 > B1) of the xylanase promoter-operator nucleotide sequence of the *Streptomyces sp.* EC3.

The Inventors have also discovered a modification of the trans factor affinity for the B2 box in repression and induction.

Repression : B3 > B2 > B1

Induction : B3 >> B2 = B1

Additional competitive experiments have identified as a preferred fixation site of the trans-activation factor according to the invention, the above-identified specific regions (boxes 3, 2 and 1).

It should be noted also that the above-described boxes present inverse repeated sequence and a palindrome of 4 BP that seems to be specifically recognised by the proteinic trans-activation factor according to the invention.

Therefore, it seems that the main fixation site of said proteinic trans-activation factor is the box B3, which allows thereafter a fixation upon the box B2 even when a mutation is present in said box B2.

According to said preliminary results, it seems that the control upon the activation of a xylanase gene is based upon operative sites which are specifically recognised by a trans-activation factor which is working as a repressor and which allows the formation of a repressive loop (connection between the B2 and B3 boxes by the trans-activation factor) and avoids the fixation of the RNA-polymerase and thereafter the transcription of a downstream coding nucleotide sequence.

Genetic identification of the proteinic trans-activation factor and its encoding nucleotide sequence

The gene coding for xylanase C of *Streptomyces sp.* has been cloned into a multicopy vector which confers positive xylanase phenotype when the host strain is under repression conditions. Repressed clones, which may be a genomic fragment encoding the repressor according to the invention, will be characterised by a wild type phenotype.

Repressors from a genomic bank in the vector pDML614 were isolated.

After plasmid purification, an amplification by PCR allows a raw estimation of the insert size, which is presented in Table 2.

PCR conditions :	Step 1 :		96 °C	4 min
	Step 2 :	30 cycles	94 °C	30 sec
	Step 3 :		54 °C	1 min
	Step 4 :		72 °C	3 min 30 sec
	Step 5 :		72 °C	10 min
	Step 6 :		4 °C	

Table 2 : Size of the insert

Clone	Size of the PCR product (kb)	Estimated size of the insert (kb)
S1	2,5	0,9
S2	3,2	2,5
S3	2,5	0,9
S4	1,6	0,1
S5	2,1	0,5
S6	2,8	1,2
pDML614	1,6	0

A sequence of 1022 nucleotides obtained from the clone S6 allows the identification of an open reading frame with several bacterial regulator systems. A first polypeptide of 164 amino acids was identified and the corresponding nucleotide sequence was used as a probe for the isolation of the complete nucleotide sequence SEQ ID NO 1.

The cloning of the carboxy terminal portion was obtained by Southern blotting. 2,5 genomic DNA of *Streptomyces sp.* EC3 are cleaved by several restriction enzymes and have been transferred upon a nylon membrane. A fragment of 720 BP has been amplified and labelled with biotine by PCR, and is used as a probe for the specific hybridisation of the genomic DNA. A portion of the genomic DNA of *Streptomyces sp.* was cleaved by restriction enzymes and the generated fragments by PCR were introduced in a plasmid pUC for sequencing.

The sequenced nucleotide sequence comprises four open reading frames. The longest open reading frame hereafter called xlnR was implicated in the regulation of the xylanase enzyme, and the corresponding amino-acid sequence was identified by the BLAST software.

The complete isolated and purified nucleotide sequence 1 according to the invention was introduced in a vector 7 having incorporated also a xylanase promoter-operator nucleotide sequence 2 linked to a gene encoding a xylanase enzyme 5. Advantageously, said xylanase promoter-operator nucleotide sequence 2 comprises a poly-linker sequence (nucleotide sequence with several cleaving sites) which improves the insertion of homologous or heterologous sequences. The characteristics of the vector according to the invention were improved by incorporating a specific marker (such as the thiostreptone) which is used for the specific selection of transformed cells.

The vector according to the invention was advantageously a shuttle vector comprising the necessary elements for the transfection of said vector in a *Streptomyces* strain and in *E. coli* (see also U.S. patent 4,992,371 incorporated hereafter by reference).

Preferably, said shuttle vector was prepared according to the method comprising the following steps. The pUC18 polylinker was replaced by a dsDNA fragment containing endonuclease restriction sites and the following dsDNA fragment was entered in a *Hin*dIII-*Eco*RI-digested pUC18 (L08752, Norrander et al., Gene 26, pp. 101-106 (1983)).

dsDNA fragment: SEQ ID NO 5: 5'- AGC TAG GCC TAT CGA TGG CGC GCC AAG CTA GCA ACT TAA GTA GAT CTA ACT AGT CTG CAG CAG AAG CTT AAT ATT TAA TTA AGC GGC CGC AGT ACT CTC GAG CCG CCA TGG GCC CGA TAT CGG TAC CAG GCC T- 3' (or SEQ ID NO 4)

(Endonuclease restriction sites: 5'-*Cla*I-*Asc*I-*Nhe*I-*Afl*II-*Bgl*II-*Spe*I-*Pst*I-*Hin*dIII-*Ssp*I-*Pac*I-*Not*I-*Sca*I-*Xho*I-*Nco*I-*Apa*I-*Eco*RV-*Kpn*I-3').

Thereafter, the streptomycine/spectinomycine resistance gene (Str/Spm) from an omega interposon

(Prentki, P. & Krisch, H.M. Gene 29 pp. 303-313 (1984)) was introduced at the *Hind*III restriction site.

The pUC18 sequence was deleted from the construction and replaced by the *Cla*I-*Kpn*I *Streptomyces* replication origin from the pIJ702 vector (Katz et al., J. Gen. Microbio. 129 pp. 2703-2714).

The construction was achieved to be a shuttle vector: a 1242 bp *Ase*I-*Nde*I DNA fragment, containing the *E. coli* DNA replication origin from the pBR322 vector (J01749, Sutcliffe, J.G., Proc. Natl. Acad. Sci. U.S.A. 75(8), pp. 3737-3741 (1978)) was treated by klenow and introduced in *Eco*RV.

The regulatory sequence *xln*R was introduced in a *Pac*I-*Sca*I-digested vector and the *xln*C structural gene with its promoter in the *Asc*I-*Pst*I restriction sites in order to obtain the shuttle vector "Vpro" according to the invention (see enclosed Fig. 3).

For the analysis of an heterologous expression of foreigner genes in *Streptomyces*, the person skilled in the art may refer to the US patent 5,641,663 and the US patent 5,435,730.

Furthermore, the vector according to the invention may also comprise one or more mutations in the xylanase promoter-operator nucleotide sequence 2 in order to improve (increase) a cis-activating by said xylanase promoter-operator nucleotide sequence.

CLAIMS

1. Isolated and purified genetic sequence (1) controlling in trans the expression of a xylanase promoter-operator nucleotide sequence (2).

2. Isolated and purified genetic sequence according to claim 1, being a nucleotide sequence which presents more than 60% homology with the nucleotide sequence SEQ ID NO 1 or its complementary strand.

3. Isolated and purified genetic sequence according to claim 2, which presents more than 80%, preferably more than 90%, more specifically more than 95%, homology with the nucleotide sequence SEQ ID NO 1 or its complementary strand.

4. Isolated and purified genetic sequence according to any one of the preceding claims, being the nucleotide sequence SEQ ID NO 1, its complementary strain or a portion thereof having more than 100 nucleotides and encoding a peptide controlling positively and/or negatively the activation of a xylanase promoter-operator nucleotide sequence.

5. Isolated and purified genetic sequence according to claim 1, being an amino-acid sequence which presents more than 60% homology with SEQ ID NO 2.

6. Isolated and purified genetic sequence according to claim 5, being an amino-acid sequence which presents more than 80%, preferably more than 90%, more specifically more than 95%, homology with SEQ ID NO 2.

7. Isolated and purified genetic sequence according to claim 1, being the amino-acid sequence SEQ ID NO 2 or a portion thereof having more than 50 amino-acids which is capable of controlling positively and/or negatively in trans the expression of a xylanase promoter-operator nucleotide sequence.

8. Nucleotide construct (6) comprising the isolated and purified nucleotide sequence according to any one of the claims 1 to 4, linked to a xylanase promoter-operator nucleotide sequence (2) and possibly a nucleotide sequence (5) which is cis-activated by said xylanase promoter-operator nucleotide sequence (2).

9. Vector (7), preferably a plasmid, comprising the isolated and purified nucleotide sequence (2) according to any one of the claims 1 to 7 or the nucleotide construct (6) according to claim 8.

10. Cell transformed by the vector according to claim 9 and which allows the expression of the isolated and purified genetic sequence according to any one of the claims 1 to 7.

SEQUENCE LISTING

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<120> NUCLEOTIDE AND/OR AMINO-ACID SEQUENCE CONTROLLING THE EXPRESSION OF A XYLANASE PROMOTER-OPERATOR NUCLEOTIDE SEQUENCE

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Ala Asp Ser Leu Arg Glu Ala Ile Gln Ser Gly Glu Tyr Gly Pro Gly
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Asp Arg Leu Pro Gly Glu Asn Asp Leu Met Ala Thr His Gly Val Ala
35 40 45

cgt atg acg gcc cgg cag gcg ctc ggc gtc ctg cgg gac gag ggc atc 312
Arg Met Thr Ala Arg Gln Ala Leu Gly Val Leu Arg Asp Glu Gly Ile
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gcc gaa tcc cgg aag ggc gca ggt gtc ttc gtg cgg gcc ttc cgt ccg 360

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Ala Glu Ser Arg Lys Gly Ala Gly Val Phe Val Arg Ala Phe Arg Pro
          65                  70                  75

ctg cgc cga cgc ggc atc cag cgg ctg gcc cgc gac cag tgg ggc aac   408
Leu Arg Arg Arg Gly Ile Gln Arg Leu Ala Arg Asp Gln Trp Gly Asn
      80                  85                  90

gga cgg tcc atc tgg tcg gcg gac atc gag gcc aga gac ctc cgg gtc   456
Gly Arg Ser Ile Trp Ser Ala Asp Ile Glu Ala Arg Asp Leu Arg Val
  95                 100                 105                 110

gac cag gtc tcg gtg ggc gag gag aaa gct ccc gag cac atc ggc gcg   504
Asp Gln Val Ser Val Gly Glu Glu Lys Ala Pro Glu His Ile Gly Ala
                 115                 120                 125

gtc ctg ggc atg gct gcc gaa gaa gtc gcg tgc gtg agg cgc cgg cgc   552
Val Leu Gly Met Ala Ala Glu Glu Val Ala Cys Val Arg Arg Arg Arg
             130                 135                 140

ttc gtc ctg gac ggc aag ccg gtg ctg ctc gcg acg agt tac ctg ccc   600
Phe Val Leu Asp Gly Lys Pro Val Leu Leu Ala Thr Ser Tyr Leu Pro
         145                 150                 155

ctg tcc ctg gtg gcc gga tcc gcc atc agc cga gag gac acc ggg ccg   648
Leu Ser Leu Val Ala Gly Ser Ala Ile Ser Arg Glu Asp Thr Gly Pro
     160                 165                 170

ggc ggt acc tac gcc cgg ctt gcc gaa ctc ggc cac gaa ccg gtg cac   696
Gly Gly Thr Tyr Ala Arg Leu Ala Glu Leu Gly His Glu Pro Val His
175                 180                 185                 190

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Phe Arg Glu Glu Ile Arg Ser Arg Met Pro Ser Pro Asp Glu Val Thr
                 195                 200                 205

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Gln Leu Asp Leu Ala Pro Gly Thr Pro Val Ile Leu Ile Cys Arg Thr
             210                 215                 220

gcg ttc acc gac cag ggc cac cct gtc gag gtc aac gag atg acc ctg   840
Ala Phe Thr Asp Gln Gly His Pro Val Glu Val Asn Glu Met Thr Leu
         225                 230                 235

gac gcc gct tcc tac gtc ttg gag tac gac ttc gac gcg ggc ccc gag   888
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Thr Ala Arg Gln Ala Leu Gly Val Leu Arg Asp Glu Gly Ile Ala Glu
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Ser Arg Lys Gly Ala Gly Val Phe Val Arg Ala Phe Arg Pro Leu Arg
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Ser Ile Trp Ser Ala Asp Ile Glu Ala Arg Asp Leu Arg Val Asp Gln
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Thr Tyr Ala Arg Leu Ala Glu Leu Gly His Glu Pro Val His Phe Arg
180 185 190

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Thr Asp Gln Gly His Pro Val Glu Val Asn Glu Met Thr Leu Asp Ala
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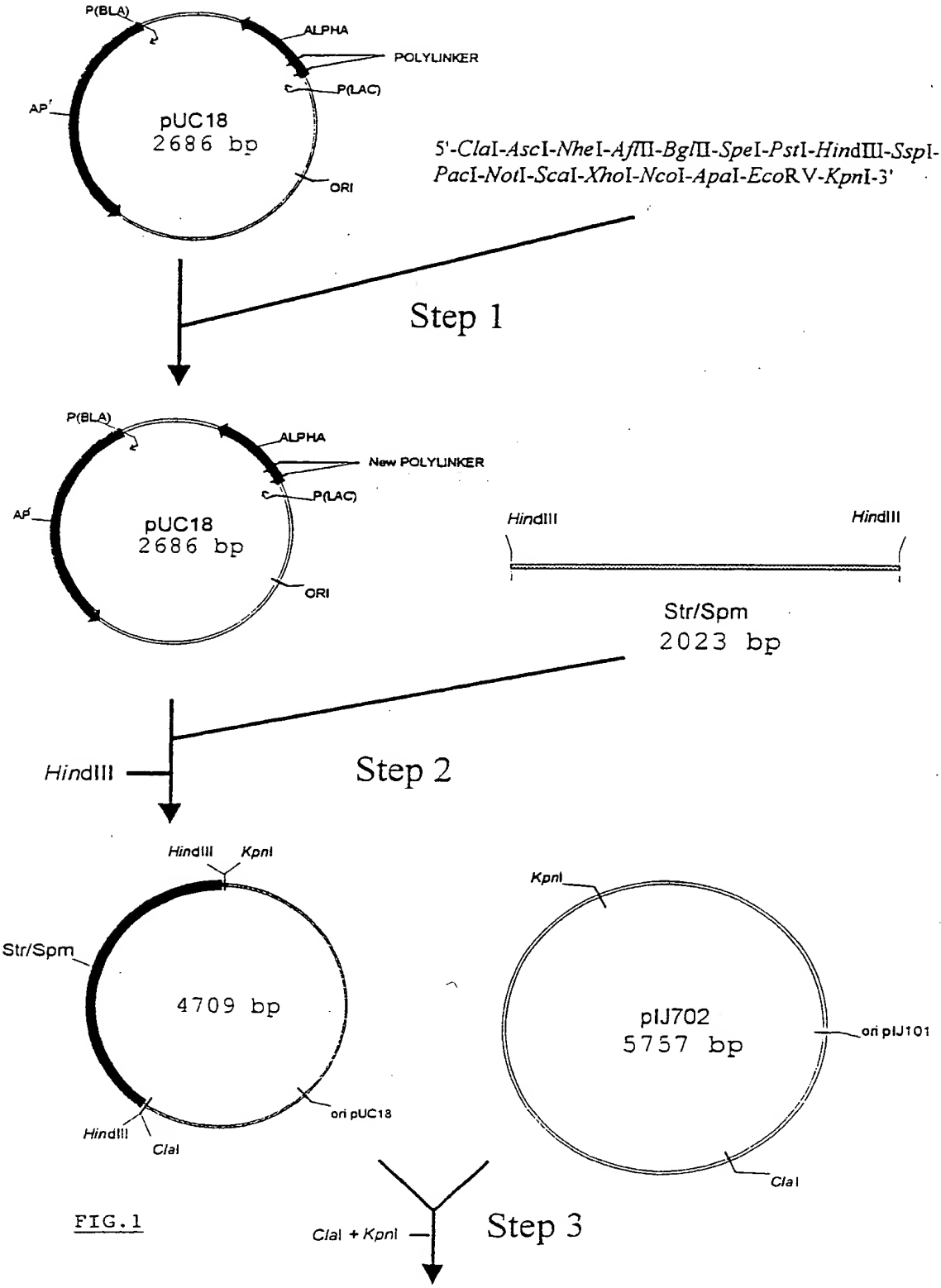
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ALPHA
POLYLINKER
P(LAC)
AP
pUC18
2686 bp
ORI
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Step 1
P(BLA)
ALPHA
New POLYLINKER
P(LAC)
AP
pUC18
2686 bp
ORI
HindIII
HindIII
Str/Spm
2023 bp
HindIII
Step 2
HindIII
KpnI
Str/Spm
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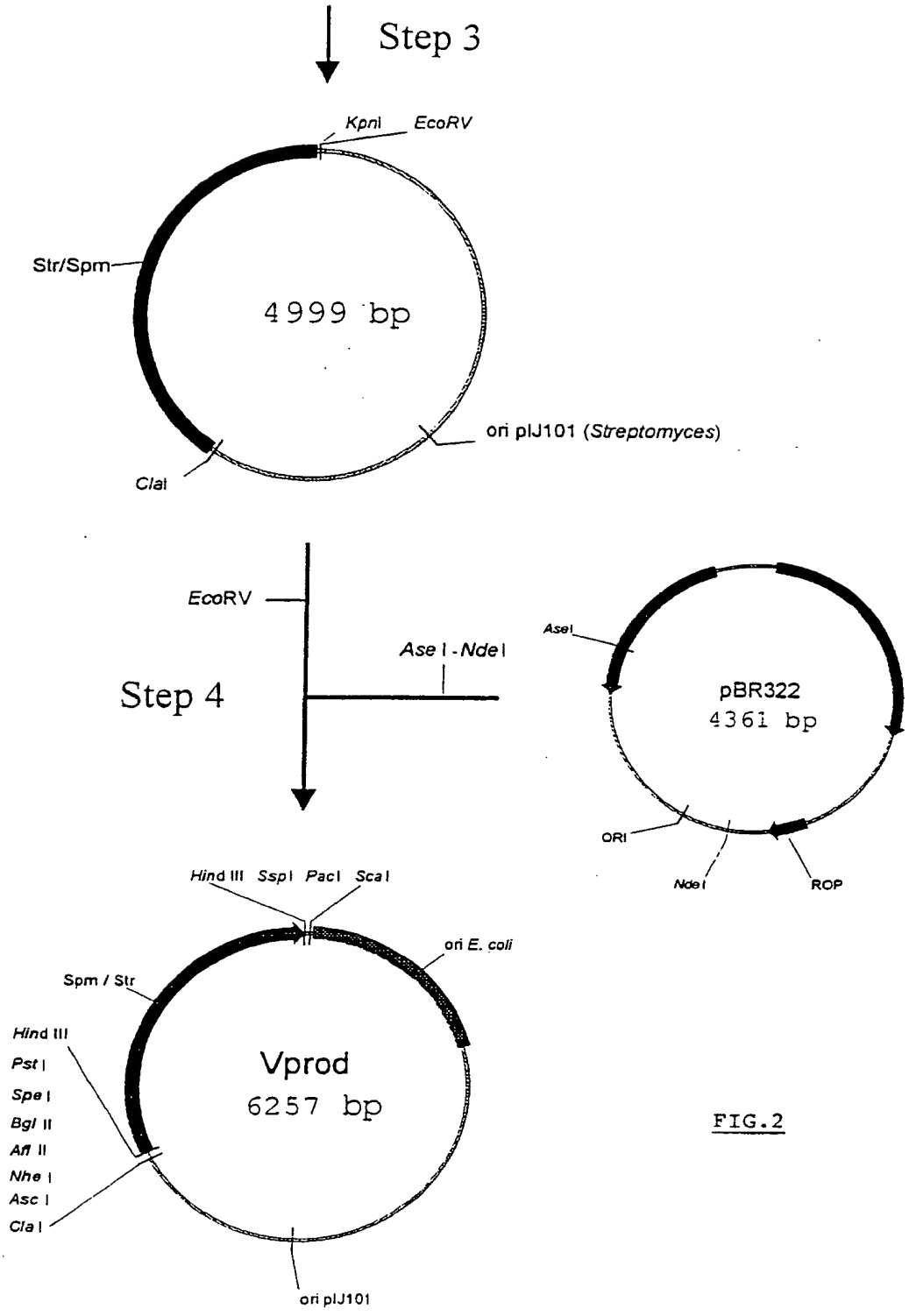
Step 3

FIG.1


Step 3
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EcoRV
Str/Spm
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ClaI
EcoRV
Ase I - Nde I
Step 4
AseI
pBR322
4361 bp
ORI
NdeI
ROP
Hind III SspI PacI ScaI
ori E. coli
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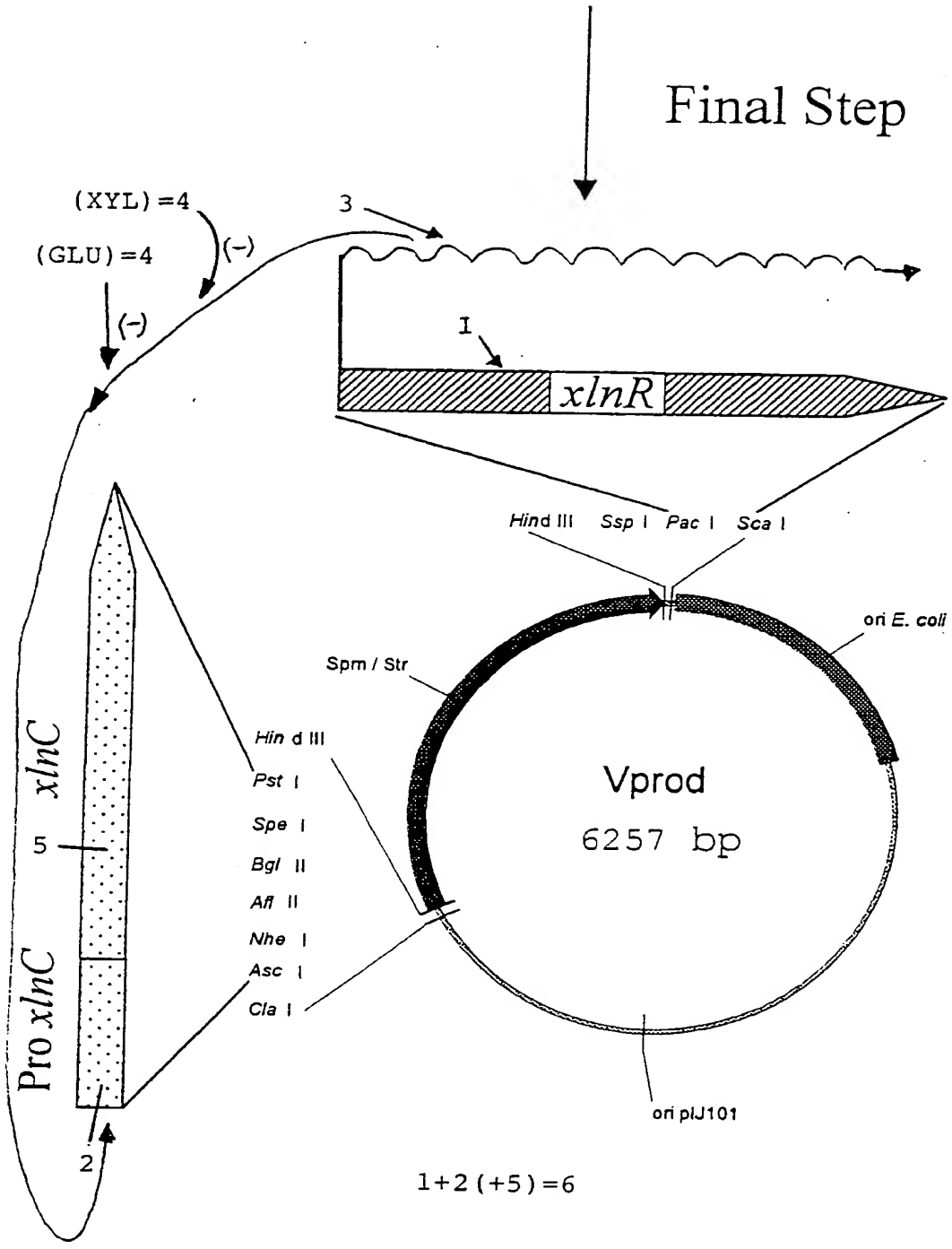
FIG.2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(GLU)=4
(-)
(-)
3
I
xlnR
Hind III
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Pac I
Sca I
ori E. coli
Spm / Str
Vprod
6257 bp
ori pIJ101
Hin d III
Pst I
Spe I
Bgl II
Afl II
Nhe I
Asc I
Cla I
5
Pro xlnC
xlnC
2
1+2(+5)=6

FIG.3

INTERNATIONAL SEARCH REPORT

International Application No
PCT/BE 99/00105

A. CLASSIFICATION OF SUBJECT MATTER
IPC 7 C12N15/76 C12N9/24 C12N1/21 C07K14/36

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)
IPC 7 C12N

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practical, search terms used)

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category °	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	VAN PEIJ N N M E ET AL: "Isolation and analysis of xlnR, encoding a transcriptional activator co-ordinating xylanolytic expression in Aspergillus niger" MOLECULAR MICROBIOLOGY., vol. 27, no. 1, January 1998 (1998-01), pages 131-142, XP000853720 OXFORD., GB	1,8-10
Y	abstract page 137	2-7

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[X] Further documents are listed in the continuation of box C.

[ ] Patent family members are listed in annex.

° Special categories of cited documents :

"A" document defining the general state of the art which is not considered to be of particular relevance

"E" earlier document but published on or after the international filing date

"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)

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"P" document published prior to the international filing date but later than the priority date claimed

"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention

"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone

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"&" document member of the same patent family

Date of the actual completion of the international search	Date of mailing of the international search report
23 November 1999	08/12/1999

Name and mailing address of the ISA	Authorized officer
European Patent Office, P.B. 5818 Patentlaan 2 NL – 2280 HV Rijswijk Tel. (+31–70) 340–2040, Tx. 31 651 epo nl, Fax: (+31–70) 340–3016	Lejeune, R

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C.(Continuation) DOCUMENTS CONSIDERED TO BE RELEVANT

Category '	Citation of document, with indication,where appropriate, of the relevant passages	Relevant to claim No.
Y	GIANNOTTA F ET AL: "A sequence-specific DNA-binding protein interacts with the xlnC upstream region of Streptomyces sp. strain EC3" FEMS MICROBIOLOGY LETTERS, vol. 142, 1996, pages 91-97, XP000853721 AMSTERDAM, NL ISSN: 0378-1097 cited in the application	2-7
A	page 91, column 2 abstract	1
A	DATABASE EMBL 'Online! Accession Nbr Z19589, 7 April 1998 (1998-04-07) HAGEGE J M: "S.ambofaciens plasmid pSAM2 gene encoding KorSA" XP002122813 56% identity in 518 BP overlap with SEQ ID 1	1-7